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Fats and oils

CARNITINE METABOLISM IN *MACACA ARCTOIDES*: THE EFFECTS OF DIETARY CHANGE AND FASTING ON SERUM TRIGLYCERIDES, UNESTERIFIED CARNITINE, ESTERIFIED (ACYL) CARNITINE, AND β-HYDROXYBUTYRATE. F.P. Bell, A. DeLucia, L.R. Bryant, C.S. Patt, H.S. Greenberg (Diabetes-Atherosclerosis Res., The Upjohn Co., Kalamazoo, MI 49001) *Am. J. Clin. Nutr. 36*(1):115-121 (1982). Serum triglycerides and serum total, esterified, and free (unesterified) carnitine were measured in 21 male *Macaca arctoides* that were switched from a low fat (5.2% w/w), high carbohydrate diet to a high fat (15.9% w/w), low carbohydrate diet for 90 days and then returned to the original low fat diet for a subsequent 76-day period. Serum triglycerides and total carnitine levels fell significantly ($p < 0.05$) during the initial 2 wk of feeding the high fat diet and the ratio of esterified/unesterified carnitine rose significantly ($p < 0.05$) on the high fat diet. A return to the low fat diet reversed these changes; triglycerides rose significantly ($p < 0.05$) within 3 days and the ratio of esterified/unesterified carnitine fell significantly ($p < 0.05$) during the same period. A return of total carnitine levels to those initially observed on the low fat diet was slower to develop. Fasting for 24 to 48 hr resulted in increases of 65 to 75% in total serum carnitine. This increase reflected elevations of both the esterified and unesterified carnitine fractions but was largely attributable to increases in esterified carnitine which rose from 10 to 41 nmol/ml by 48 hr while unesterified carnitine rose from 55 to 72 nmol/ml during the same period. In addition, the ratio of esterified/unesterified carnitine rose from 0.183 ± 0.023 to 0.583 ± 0.069 (n=8) with a 48-hr fast and was significantly correlated with serum β-hydroxybutyrate levels at both 24 and 48 hr.

EVIDENCE FOR PROTEIN-ASSOCIATED LIPIDS FROM DEUTERIUM NUCLEAR MAGNETIC RESONANCE STUDIES OF RHODOPSIN-DIMYRISTOYLPHOSPHATIDYLCHOLINE RECOMBINANTS. A. Bienvenue, M. Bloom, J.H. Davis, and P.F. Devaux (Laboratoire de Biologie Physio-Chimique, Universite des Sciences et Techniques du Languedoc, 34060 Montepellier, Cedex, France) *J. Biol. Chem. 257*(6):3032-3038 (1982). The technique of deuterium magnetic resonance was, used to study the orientational order of the perdeuterated acyl chains of dimyristoylphosphatidylcholine (DMPC-d_{54}) reconstituted with rhodopsin between 0 and 23 C. This range includes the gel to liquid crystalline phase transition of DMPC-d_{54} at 20 C. Various molar lipid protein (L/P) ratios were investigated. Measurements of orientational order parameters showed that the addition of rhodopsin broadened the range of the gel to liquid crystalline transition for L/P=150 and 50. No transition was observed for L/P=30 and 12. Moment analysis and spectral subtraction both showed that the low temperature spectra for L/P>30 had two components. One was a pure phospholipid gel phase spectrum and the other a spectrum attributed to lipids in protein aggregates. The intensity of the second component corresponded to 30 lipids/protein and its shape was the same as the temperature-independent shape observed for L/P=30 and 12. No such decomposition into two components was possible in the liquid crystalline phase for L/P>30. Extraction of the oriented ^{2}H NMR spectrum from its powder spectrum showed that the presence of proteins does not modify the distribution of quadrupole splittings but does produce both homogeneous and inhomogeneous broadening. The latter may be related to the heterogeneity seen in the spectra of electron paramagnetic resonance spin labels above the gel to liquid crystalline transition.

ASSESSMENT OF ABDOMINAL FAT CONTENT BY COMPUTED TOMOGRAPHY. G.A. Borkan, S.G. Gerzof, A.H. Robbins, D.E. Hults, C.K. Silbert, and J.E. Silbert (Normative Aging Study, Veterans Admin. Outpatient Clinic, Boston, Massachusetts and Dept. of Radiology and Med., Veterans Admin. Med. Center, Boston, MA) *Am. J. Clin. Nutr. 36*(1):172-177 (1982). Computed tomography (CT) produces thin cross-sectional radiographs that may prove very useful in body composition research. CT images of the abdomen allow computerized measurement of total fat area, and also enable the differentiation of subcutaneous fat from intraabdominal fat. The present investigation examines whether a single CT scan of the abdomen provides an accurate indication of overall abdominal adiposity. Graphs of measurements from seven sequential scans of the abdomen in eight patients showed that rankings of total abdominal area, total fat area, subcutaneous and intraabdominal fat area are relatively consistent no matter which abdominal level is chosen. Correlations of 0.89 and 0.99 between single scans and the average values for all scans show that a single CT image contains the same information on adiposity as a series of scans. These results suggest that future CT studies of body composition can limit radiation exposure by using single scans at different anatomical sites. If only a single scan at one site can be obtained, the level of the umbilicus may be the most useful, because it contains the largest percentage of fat in the body, and best allows differentiation of intraabdominal from subcutaneous fat.

PURIFICATION AND PARTIAL CHARACTERIZATION OF A BACTERIAL PHOSPHOLIPID: CHOLESTEROL ACYLTRANSFERASE. J.T. Buckley, L.N. Halasa, and S. MacIntyre (Dept. of Biochem. and Microbio., Univ. of Victoria, Victoria, British Columbia, VSW 2Y2 Canada) *J. Biol. Chem. 257*(6):3320-3325 (1982). A glycerophospholipid:cholesterol acyltransferase has been purified to near homogeneity from cell-free culture supernatants of *Aeromonas salmonicida*. The characteristics of the enzyme distinguish it from bacterial phospholipases; however, it shares several properties with the lecithin:cholesterol acyltransferase of mammalian plasma. Thus, the enzyme exhibits 2-positional specificity as an acyltransferase and it will act as a phospholipase A_2 in the absence of cholesterol. Furthermore, it has no divalent cation requirement and it is stimulated both by albumin and by human apolipoprotein A-I. Unlike the mammalian acyltransferase, however, the bacterial enzyme is not specific for phosphatidylcholine and in addition it can use human erythrocyte membranes as substrates. Similar to *Naja naja* phospholipase A_2, it acts asymmetrically on intact erythrocytes.

EFFECT OF DIETARY EGGS AND ASCORBIC ACID ON PLASMA LIPID AND LIPOPROTEIN CHOLESTEROL LEVELS IN HEALTHY YOUNG MEN. I.M. Buzzard, M.R. McRoberts, D.L. Driscoll, J. Bowering (Dept. of Human Nutr., Syracuse Univ., Syracuse, NY 13210) *Amer. J. Clin. Nutr. 36*(1):94-105 (1982). The effects on plasma lipid levels of increased ingestion of whole eggs and of ascorbic acid (AA) were investigated in 40 healthy, free-living men, aged 21 to 35 yr. Ten subjects were assigned to each of four groups which included the following regimens in combination with the usual daily diet: EGGS group, 3 eggs + placebo; AA group, 2 g ascorbic acid; EGAA group, 3 eggs + 2 g ascorbic acid; CONTROL group, placebo only. Fasting blood was sampled at 2-wk intervals during a 6-wk experimental period and during a 4-wk postexperimental period. Dietary cholesterol intake increased from a mean (±SD) of 412 (±200) mg/day during the preexperimental period to 975 (±134) mg/day on the experimental egg diet ($p<0.001$). Mean changes in plasma lipids in the EGGS and AA groups were not statistically significant. Considerable variability in individual responses was observed. In the EGAA group, significant increases in total cholesterol (18.3 ± 6.4 mg/dl) and low-density lipoprotein cholesterol (10.0 ± 6.4 mg/dl) at 4 wk were observed. Analysis of variance indicated significant interaction between the effects of eggs and AA. A possible synergistic relationship between these two dietary factors is suggested.

STEAROYL-COENZYME A DESATURASE ACTIVITY IN THE MAMMARY GLAND AND LIVER OF LACTATING RATS. M.A. Calabro, M. Renuka Prasad, S.J. Wakil, and V.C. Joshi (Marrs McLean Dept. of Biochem., Baylor College of Med., Houston, TX 77030) *Lipids 17*(6):397-402 (1982). Stearoyl-CoA desaturase activity in microsomes from lactating rat mammary gland is very low (0.05-0.15 nmol/min/mg of protein) regardless of lactating time. In such microsomes, reductase activities and content of cytochrome b_5 are several-fold lower than in normal rat liver microsomes. Preincubation of the mammary microsomes are several-fold lower than in normal rat liver microsomes. Preincubation of the mammary microsomes with purified terminal desaturase gives a 55-fold stimulation of stearoyl-CoA desaturase activity, whereas preincubation with cytochrome b_5 has no effect. However, preincubation of mammary microsomes with both cytochrome B_5 and terminal desaturase results in a 200-fold stimulation of overall desaturation. These observations suggest that negligible stearoyl-CoA desaturase activity in lactating rat mammary microsomes is due to a low cytochrome b_5 content and the absence of terminal enzyme. The hepatic stearoyl-CoA desaturase activity increases 9-fold during lactation. There is little or no change in the NADH-cytochrome c reductase activity or in the concentration of cytochrome b_5 during this period, but the activity of the terminal desaturase increases with the increase of overall desaturation. These results suggest that liver is one of the more important sources of oleic acid for milk triglycerides.

DIRECT ACTIVATION OF CALCIUM-ACTIVATED, PHOSPHOLIPID-DEPENDENT PROTEIN KINASE BY TUMOR-PROMOTING PHORBOL ESTERS. M. Castagna, Y. Takai, K. Kaibuchi, K. Sano, U. Kikkawa, Y. Nishizuka (Dept. of Biochem., Kobe Univ. Schl. of Med., Kobe 650 and the Dept. of Cell Biol., Natl. Inst. for

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Basic Biol., Okazaki 444, Japan) *J. Biol. Chem. 257*(13):7847-7851 (1982). Tumor-promoting phorbol esters such as 12-O-tetradecanoyl-13-acetate (TPA) directly activate *in vitro* Ca^{2+}-activated, phospholipid-dependent protein kinase (protein kinase C), which normally requires unsaturated diacylglycerol. Kinetic analysis indicates that TPA can substitute for diacylglycerol and greatly increases the affinity of the enzyme for Ca^{2+} as well as for phospholipid. Under physiological conditions, the activation of this enzyme appears to be linked to the receptor-mediated phosphatidylinositol breakdown which may be provoked by a wide variety of extracellular messengers, eventually leading to the activation of specific cellular functions or proliferation. Using human platelets as a model system, TPA is shown to enhance the protein kinase C-specific phosphorylation associated with the release reaction in the total absence of phosphatidylinositol breakdown. Various phorbol derivatives which have been shown to be active in tumor promotion are also capable of activating this protein kinase in in vitro systems.

DIGITONIDE PRECIPITABLE STEROLS: A REEVALUATION WITH SPECIAL ATTENTION TO LANOSTEROL. R.J. Cenedella (Dept. of Biochem., Kirksville College of Osteopathic Med., Kirksville, MO 63501) *Lipids 17*(6):443-447 (1982). The ability of digitonin to precipitate lanosterol from prepared mixtures and biological sources was evaluated. Commercially available lanosterol was determined to be composed of about 60% lanosterol and 40% dihydrolanosterol. Both sterols were only partially precipitated by digitonin under all conditions examined. The presence of cholesterol increased the precipitation of lanosterol, but never to completion. About 40% of the lanosterols from saponified sheep's-wool fat was not precipitated by digitonin. Also ^{14}C-labeled lanosterol recovered from rat brain following intracerebral injection of 2-[^{14}C]mevalonate was only 70% precipitated by digitonin. Steric hinderance by the methyl groups at carbon -4 is suggested to explain the poor precipitability of this sterol. In conclusion, lanosterol can not be considered to be a digitonide-precipitable sterol equivalent to cholesterol. Caution should be exercised in situations where digitonin-precipitable sterols are being prepared from sources containing significant concentrations of lanosterol (i.e., mass and/or radiolabel).

DEFECTIVE *IN VITRO* LIPOLYSIS OF TYPE IV HYPERLIPIDEMIC HUMAN PLASMA BY PURIFIED MILK LIPOPROTEIN LIPASE. STUDIES BY SINGLE VERTICAL SPIN CENTRIFUGATION. B.H. Chung, J.T. Cone, and J.P. Segrest (Depts. of Pathology, Biochem. and Microbiol., and Comprehensive Cancer Center, Univ. of Alabama in Birmingham Med. Center, Birmingham, AL 35294) *J. Biol. Chem. 257*(13):7472-7480 (1982). *In vitro* interactions of purified lipoprotein lipase with plasma from type IV hyperlipidemic and normolipidemic subjects were studied using an automated cholesterol analysis adaptation to single vertical spin centrifugation. We conclude that: 1) VLDL and, to a lesser extent, VLDL infranatant from triglyceride-rich type IV hyperlipidemic subjects are defective in their interactions with bovine milk lipoprotein lipase and 2) these defects seem certain to be related to and may, in fact account for the abnormal plasma lipoprotein profiles characteristic of these subjects.

THE COVALENT STRUCTURE OF APOLIPOPROTEIN A-I FROM CANINE HIGH DENSITY LIPOPROTEINS. H. Chung, A. Randolph, I. Reardon, and R.L. Heinrikson (Dept. of Biochem., The Univ. of Chicago, Chicago, IL 60637) *J. Biol. Chem. 257*(6):2961-2967 (1982). The complete amino acid sequence of apolipoprotein A-I (apo-A-I) from canine serum high density lipoproteins (HDL) has been determined by automated Edman degradation of the intact protein and proteolytic fragments derived therefrom. The major strategy involved analysis of overlapping sets of peptides generated by cleavage at lysyl residues with *Myxobacter* protease and by tryptic hydrolysis at arginines in the citraconylated protein derivative. Canine apo-A-I has 232 residues in its single polypeptide chain and its covalent structure is highly homologous to one of the two reported sequences for human apo-A-I. As in the case for the human apoprotein, predictive analysis of the canine apo-A-I sequence suggests that it comprises a series of amphiphilic α helices punctuated by a periodic array of prolyl residues. Human HDL contains a second major protein component, apolipoprotein A-II(apo-A-II) that is lacking in HDL from dog serum. The absence of apo-A-II in canine HDL raised the possibility that the apo-A-I from this source might contain within its primary structure sequences related to apo-A-II and thus perform the dual function of both proteins in one. Our analysis proves that canine apo-A-I has all of the structural features of human apo-A-I and that it is not an A-I: A-II hybrid molecule.

SUPPRESSION OF RAT LIVER FATTY ACID SYNTHESIS BY EICOSA-5,8,11,14-TETRAYNOIC ACID WITHOUT A REDUCTION IN LIPOGENIC ENZYMES. B.A. Clarke, S.D. Clarke (Dept. of Food Sci. and Nutr., Univ. of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108) *J. Nutr. 112*(6):1212-1219 (1982). In meal-fed rats supplementation of safflower oil (5 g per 100 g diet) to a fat-free basal diet resulted in the characteristic suppression of liver fatty acid synthetase and acetyl-CoA carboxylase activities, which was accompanied by a 60% decrease in the rate of hepatic fatty acid synthesis. The decline in activity of these lipogenic enzymes was completely prevented by adding 0.05% eicosa-5,8,11,14-tetraynoic acid (TYA) to the safflower oil diet. Fatty acid analysis of the livers indicated that TYA significantly impaired the conversion of linoleate to arachidonate. Apparently the selective suppression of lipogenic enzymes by dietary linoleate is not caused by linoleate per se but requires its conversion to longer-chain fatty acids and/or prostaglandins. In spite of high activities of fatty acid synthetase and acetyl-CoA carboxylase, liver fatty acid synthesis continued to be inhibited by the safflower oil + TYA dietary regimen. This continued inhibition of lipogenesis was due to the TYA, because addition of TYA to the fat-free diet precipitated a significant decline in liver fatty acid synthesis without a drop in lipogenic enzymes. Inhibition of fatty acid synthesis by TYA could not be attributed to a decrease in liver glucose utilization based on hepatic glycogen concentration, nor was it due to a reduction in the fraction of catalytically active polymeric acetyl-CoA carboxylase based on sensitivity of the enzyme activity to avidin.

THE DISCOVERY OF A LIPID-LINKED GLUCURONIDE AND ITS SYNTHESIS BY CHICKEN LIVER. R.D. Cummings and S. Roth (The Johns Hopkins Univ., Dept. of Bio., Baltimore, MD 21218) *J. of Biol. Chem. 257*(4):1755-1764 (1982). Upon incubation with uridine diphosphate-[^{14}C]glucuronic acid, membrane fractions from adult and phenobarbital-induced embryonic liver synthesize a single glucuronide, which is soluble in chloroform:methanol (2:1). The compound is completely hydrolyzed and glucuronic acid released by either mild acid or β-glucuronidase, whereas mild base hydrolysis results in a mixture of glucuronic acid and glucuronic acid-1,2-cyclic phosphate. These data and the behavior of the lipid-linked glucuronide on DEAE-cellulose chromatography indicate that the compound contains a monophosphate diester of glucuronic acid, which is β-linked to a lipid. The synthesis of the lipid-linked glucuronide in uninduced normal embryonic liver is very low at all developmental ages up to hatching, but the introduction of phenobarbital into the air space of a 9-10-day-old embryo causes a premature increase of activity within 7 days. The glucuronyltransferase in adult and induced embryonic liver has a K_m for UDPGLcUA of 0.17×10^{-3} M and a broad pH optimum between pH 6 and 7. Glucuronic acid is released from the lipid-linked glucuronide by a β-glucuronidase in liver that is active at neutral pH and is not inhibited by saccharolactone. This glycosidase activity appears to be distinct from the previously characterized lysosomal β-glucuronidase. Fractionation of adult chicken liver membranes by differential centrifugation indicates that over 70% of the glucuronyl-transferase is associated with the nuclear and mitochondrial fractions. The endogenous β-glucuronidase capable of hydrolyzing the lipid-linked glucuronide was not separated from the glucuronyl-transferase activity during fractionation.

EFFECT OF ALTERED NEONATAL NUTRITION ON THE DEVELOPMENT OF ENZYMES OF LIPID AND CARBOHYDRATE METABOLISM IN THE RAT. D.A. Duff and K. Snell (Div. of Biochem., Dept. of Biochem., Univ. of Surrey, GU2 5XH U.K.) *J. Nutr. 112*(6):1057-1066 (1982). Rats were overnourished during suckling by litter-size manipulation in order to investigate the possible association of overfeeding in infancy with the development of obesity in later life. Rats were raised in litters of 4, 10, and 16 corresponding to overfeeding, normal feeding and underfeeding during the suckling period. From 6-19 days post partum, growth rates of pups from different litter sizes were significantly different (4>10>16). Differences in mean body weights between the groups continued to increase after weaning when all groups were allowed access to diet ad libitum and the significant weight difference between overfed and normally fed rats persisted into adult life in both males and females. Overfed animals showed modifications in the development of activities of a number of hepatic enzymes involved in lipid and carbohydrate metabolism. In later life (20 weeks) neonatally overnourished rats exhibited alterations in hepatic enzyme activities that reflected an increased capacity for lipid synthesis by the liver. "Supernourishment" of neonatal rats (by incubation with glucose of animals in small litters), accelerated the appearance of some of these alterations. These studies show that the pattern of early infant nutrition can profoundly influence the activities of liver enzymes in later, adult life.